

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY


(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 13 JUN 2006

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| Applicant's or agent's file reference P26205WOMRM | FOR FURTHER ACTION | | See Form PCT/IPEA/416 |
| International application No. PCT/GB2005/001056 | International filing date (day/month/year) 22.03.2005 | Priority date (day/month/year) 22.03.2004 | |
| International Patent Classification (IPC) or national classification and IPC INV. C12Q1/22 C12Q1/48 | | | |
| Applicant HEALTH PROTECTION AGENCY et al. | | | |
| <p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> <i>sent to the applicant and to the International Bureau</i> a total of 12 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> <i>(sent to the International Bureau only)</i> a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p> | | | |
| <p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p> | | | |
| Date of submission of the demand 26.09.2005 | | Date of completion of this report 13.06.2006 | |
| Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 | | Authorized officer Vadot-Van Geldre, E Telephone No. +31 70 340-1973 | |



INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/001056

Box No. I Basis of the report

1. With regard to the **language**, this report is based on
- ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3(a) and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))
2. With regard to the **elements*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

Description, Pages

| | |
|-----------|--|
| 1-5, 7-71 | as originally filed |
| 6 | received on 08.05.2006 with letter of 05.05.2006 |

Sequence listings part of the description, Pages

| | |
|------|---------------------|
| 1-38 | as originally filed |
|------|---------------------|

Claims, Numbers

| | |
|------|--|
| 81 | received on 20.01.2006 with letter of 19.01.2006 |
| 1-80 | received on 08.05.2006 with letter of 05.05.2006 |

Drawings, Sheets

| | |
|---------|---------------------|
| 1/9-9/9 | as originally filed |
|---------|---------------------|

- ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2005/001056

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|-------------------------------|-------------|------------------|
| Novelty (N) | Yes: Claims | 1-16,29-33,35-80 |
| | No: Claims | 17,18,19,28,34 |
| Inventive step (IS) | Yes: Claims | 35-80 |
| | No: Claims | 1-34 |
| Industrial applicability (IA) | Yes: Claims | 1-80 |
| | No: Claims | |

2. Citations and explanations (Rule 70.7):

see separate sheet

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2005/001056

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ on paper
 - ☒ in electronic form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in electronic form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment* on
 2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
 3. Additional comments:
- * *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Reference is made to the following documents :

D1 : WO2004/003226

D2 : WO0046357

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

5.1. The subject-matter of claim 17-18 does not meet the criteria of Article 33(2) PCT having regard to novelty and Article 33(3) PCT with regard to inventive step.

5.1.1. The subject-matter of independent claims 17-18 pertains to specific thermostable kinases as identified by their sequence IDs. All these sequences have been genetically engineered to provide improved stability or to optimize the expression in E. coli. The proteins encoded by a nucleic acid sequence selected from SEQ ID 27, 29 or 30 are genetically engineer to optimize the expression in E. coli and will thus have the same protein sequence as the natural thermostable kinase. Indeed, the codon usage of these DNA sequences has been optimized for expression in E. coli, but this results in the same protein. The thermostable adenylate kinase from Sulfolobus acidocaldarius, Thermotoga maritima and Archaeoglobus fulgidus were already well-known in prior art. Indeed, the contribution of the present application does not reside in the cloning of new thermostable kinases, as was implicitly admitted by the applicant in the introduction of the description and is also clear from eg. DNA or protein databases at EBI or NCBI (eg. CAA51967, NP_229279, AAB90565). Consequently, claims 17 and 18 lack novelty.

5.1.2. The subject-matter of claims 28 and 34 pertaining to a kit comprising the thermostable kinase and a substrate therefor, also lack novelty for the same reasons.

5.1.3. The dependent claims 29-33 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the EPC with respect to inventive step, as the relevant subject matter is either disclosed in the cited prior art or also falls within the knowledge and ability of the skilled person

5.1.4. As a matter of completeness for claims 17-18 and with regard to SEQ IDs 17-19,

it is specified that none of the prior art documents cited on the search report disclose or hint at the genetical engineering of thermostable kinases in order to even further improve their stability.

5.2. The subject-matter of claims 19-27 is not novel and/or does not involve an inventive step (Art. 33(2) and (3) PCT).

5.2.1. D1 (pg 12, l 29-31 ; pg 10, l 24 - pg 11, l 2) an indicator comprising a thermostable kinase coated upon a solid support such as a bead. Although D1 requires the presence of a seal, the subject-matter of claim 19 is not limited to non sealed indicators. D1 thus anticipates the subject-matter of claims 19.

5.2.2. The independent claims 28 and 34 and the dependent claims 20-27, 29-33 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the EPC with respect to inventive step, as the relevant subject matter is either disclosed in the cited prior art or also falls within the knowledge and ability of the skilled person

5.3. The subject-matter of claims 35-80 meets the criteria of Article 33(2) and (3) PCT having regard to novelty and inventive step.

5.3.1. A method of validation a treatment process involving the use of a thermostable kinase as an indicator for a treatment process as defined in claim 35, wherein the treatment process also acts directly on the thermostable kinase and the kinase thus undergoes the same treatment process as the biological agent was not disclosed in prior art.

5.3.2. D1 describes the use of an enzyme as a biological indicator for validating the effect of a thermal treatment process on the amount or activity of a biological agent in a sample. The enzyme is present within a sealed container so as to provide at least one moisture vapor barrier (D1 : claim 1) The subject-matter of claim 35 differs therefrom in that a thermostable kinase is used and that the thermostable kinase also undergoes the treatment process independently from the treatment process. The effect thereof is that the treatment process (eg. pH, chemical sterilant,...) can be validated over a broad dynamic range well beyond 6 logs, to 8 logs and more thus increasing the scope of monitoring (pg 4, last par). The problem solved by the subject-matter of claim 1 can therefore be formulated as how to provide an

alternative biological indicator for validating a treatment process for reducing the amount or activity of a biological agent in a sample, said indicator allowing validation over a broad dynamic range well beyond 6 logs, to 8 logs. The solution, perform the treatment process in the presence of the thermostable kinase, so that the treatment process also acts directly on the kinase and the kinase undergoes the same treatment conditions, was not suggested in the prior art.

5.3.3. None of the prior art documents suggest or teach the use of a thermostable kinase as an indicator for validating treatment processes. D1 furthermore leads the skilled person away from providing a direct contact between the enzyme and the treatment process, because it describes the seal as essential for efficient working of the enzyme (pg 11, l 14-18).

5.4 The applicant argued inter alinea that the fact that the kinase is directly exposed to the treatment process is an inherrent feature of claims 1, because for the remaining treatment processes to be able to act on the kinase, there must be a direct contact between these processes and the kinase. However, the direct exposure of the kinase to the treatment process only becomes inherrent when the treatment process (as defined in claim 1) is performed in the presence of the thermostable kinase (as said in claim 35). However, this feature, namely "the treatment process in the presence of the thermostable kinase" is not present in claims 1. Claim 1 thus only requires that a treatment process is carried out for decontamination and that a thermostable kinase is used to validate whether the decontamination was successfull. As a result, in the absence of this feature from said claims, the presence of an inventive step has to be denied. Indeed, both D1 (pg 12, l 21-31 ; pg 17, l 6-11 ; claims) and D2 (example 1 ; pg 3, l 15-32 ; pg 9, l 16-25 ; pg 13, l 3-25) disclose the use of a thermostable kinase to validate decontamination. The subject-matter of claim 1 only differs from D1 or D2 in that other treatment processes than temperature are used for decontamination. This represents however a mere choice from the well-known alternatives which are available for temperature when decontamination is required. The substitution of temperature treatment with eg. pressure as treatment process also does not involve a technical prejudice which has to be overcome. Consequently, claims 1 as well as the claims dependent thereon (claim 2-16) do not involve an inventive step (Art. 33(3) PCT)

5.5. The claims meet the criteria of Article 33(4) PCT with regard to industrial applicability.

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/GB2005/001056

Re Item V

Certain observations on the international application

1. In order to make clear (Art. 6 PCT) that also the sample containing the kinase is subjected to the treatment process in claim 74, it is necessary to replace "sample(s)" with "samples" in step (ii).

CLAIMS

1. Use of a thermostable kinase as an indicator for validating a treatment process for reducing the amount or activity of a contaminating biological agent in a sample;

wherein the treatment process comprises exposure to one or more of a selected pH, pressure, enzyme, detergent, chemical sterilant or gas-phase sterilant;

and wherein the biological agent is selected from the group consisting of bacteria, viruses, spores, proteins, peptides and prions.

2. Use according to Claim 1, wherein kinase is immobilised in or immobilised on a solid support.

3. Use according to Claim 1 or 2, wherein the kinase is adenylate kinase, acetate kinase or pyruvate kinase.

4. Use according to any of Claims 1-3, wherein the kinase catalyses formation of ATP from a substrate comprising ADP.

5. Use according to any of Claims 2-4, wherein the solid support is a matrix and the kinase is dispersed within the matrix.

6. Use according to Claim 5, wherein the solid support comprises a polymer matrix.

7. Use according to any of Claims 2-6, wherein the solid support is an indicator strip, a dip stick or a bead.

8. Use according to any of Claims 1-7, wherein the indicator further comprises an agent to stabilise the kinase.

9. Use according to Claim 8, wherein the stabilising agent is selected from metal ions, sugars, sugar alcohols and gel-forming agents.
10. Use according to any of Claims 2-9, further comprising means to attach the support to a surface.
11. Use according to Claim 10, comprising a projection, recess or aperture for attachment of the support to a surface by means of a screw, nut and bolt or clamp.
12. Use according to any preceding claim, wherein the biological agent is an infectious biological agent and the treatment is for reducing the infectious activity of the agent.
13. Use according to any preceding claim, wherein the biological agent is a transmissible spongiform encephalopathy.
14. Use according to any preceding claim, wherein the treatment process comprises one or more of, high temperature, high pH, high pressure, exposure to a protease, exposure to a detergent, a chemical sterilisation treatment or a gas-phase sterilisation treatment.
15. Use according to any preceding claim, wherein the treatment process comprises exposing the sample to a thermostable protease at a temperature of at least 60 degrees C and at a pH of at least 9.
16. Use according to any preceding claim, wherein the kinase has an amino acid sequence selected from the group consisting of SEQ ID Nos: 1-25 or is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 26-30.

17. A biological process indicator comprising a thermostable kinase, wherein said kinase has an amino acid sequence selected from the group consisting of SEQ ID Nos: 17-19 or is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 27, 29 and 30.

18. A thermostable kinase having an amino acid sequence selected from the group consisting of SEQ ID Nos: 17-19, or encoded by a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 27, 29 and 30.

19. A biological process indicator comprising a thermostable kinase that retains at least 95% activity after exposure to 70 °C for 30 minutes, and a solid support selected from the group consisting of an indicator strip, a dip stick, a bead or a matrix, wherein the kinase is immobilised in the solid support, or is immobilised on the solid support by chemical cross-linking or adsorption.

20. A biological process indicator according to Claim 17 or 19, wherein the kinase catalyses formation of ATP from a substrate comprising ADP.

21. A biological process indicator according to Claim 19 or 20 wherein the solid support is a matrix and the kinase is dispersed within the matrix.

22. A biological process indicator according to Claim 21, wherein the support comprises a polymer matrix.

23. A biological process indicator according to Claim 19 or 20, wherein the support is an indicator strip, a dip stick or a bead.

24. A biological process indicator according to any of Claims 19-23, further comprising an agent to stabilise the kinase.

25. A biological process indicator according to Claim 24, wherein the stabilising agent is selected from metal ions, sugars, sugar alcohols and gel-forming agents.

26. A biological process indicator according to any of Claims 19-25, further comprising means to attach the indicator to a surface.

27. A biological process indicator according to Claim 26, comprising a projection, recess or aperture for attachment of the indicator to a surface by means of a screw, nut and bolt or clamp.

28. A kit for use in validating a treatment process for reducing the amount or activity of a biological agent in a sample comprising:

- (i) a biological process indicator according to any of Claims 17 or 19-27 and
- (iii) substrate for the kinase.

29. A kit according to Claim 28, further comprising means for detecting ATP.

30. A kit according to Claim 29, further comprising luciferin/luciferase.

31. A kit according to any of Claims 28-30, further comprising a luminometer.

32. A kit according to any of Claims 28-31, further comprising a look-up table correlating the kinase activity of the indicator with the reduction in the amount or activity of the biological agent.

33. A kit according to any of Claims 28-32, for monitoring TSE inactivation.

34. A portable kit according to any of Claims 28-33.

35. A method of validating a treatment process, comprising:

- (i) obtaining a sample that contains, or is suspected to contain, a contaminating biological agent, wherein the biological agent is selected from the group consisting of bacteria, viruses, spores, proteins, peptides and prions;
- (ii) subjecting the sample to a treatment comprising exposure to one or more of a selected pH, pressure, enzyme, detergent, chemical sterilant or gas-phase sterilant, in the presence of a defined amount of a thermostable kinase; wherein the treatment reduces the amount or activity of the biological agent;
- (iii) measuring residual kinase activity and optionally calculating the reduction in kinase activity; and
- (iv) comparing said residual activity to a predetermined kinase activity, or comparing said reduction in kinase activity to a predetermined reduction in kinase activity, wherein the predetermined kinase activity or predetermined reduction in kinase activity corresponds to a confirmed reduction in the amount or activity of the biological agent under the same treatment conditions.

36. A method according to Claim 35, wherein the sample is known to contain the biological agent.

37. A method according to Claim 35 or 36, wherein the biological agent is an infectious biological agent, and the treatment reduces the infectious activity of the biological agent.

38. A method according to any of Claims 35-37, wherein the biological agent is a transmissible spongiform encephalopathy.

39. A method according to any of Claims 35-38, wherein the kinase is a thermostable kinase.

40. A method according to any of Claims 35-39, wherein the kinase is an adenylate kinase, an acetate kinase or a pyruvate kinase.

41. A method according to any of Claims 35-40, wherein the kinase has an amino acid sequence selected from the group consisting of SEQ ID Nos: 1-25 or is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 26-30.

42. A method according to any of Claims 35-41, wherein the treatment comprises one or more of high temperature, high pH, high pressure, exposure to a protease, exposure to a detergent or a chemical sterilant.

43. A method according to Claim 42, wherein the treatment comprises exposing the sample to a thermostable protease at a temperature in the range 50-120°C.

44. A method according to Claim 43, wherein the treatment comprises exposing the sample to the protease at a temperature of 60°C or above.

45. A method according to Claim 44, comprising exposing the sample to the protease at a pH of 9 or above.

46. A method according to any of Claims 35-45, wherein the kinase, prior to the treatment, has an activity of at least 10,000,000 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

47. A method according to Claim 46, wherein the kinase, prior to the treatment, has an activity of at least 5,000,000 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

48. A method according to Claim 47, wherein the kinase, prior to the treatment, has an activity of at least 1,000,000 Relative Light Units per mg

kinase when measured in the presence of luciferin/luciferase by a luminometer.

49. A method according to Claim 48, wherein the kinase, prior to the treatment, has an activity of at least 500,000 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

50. A method according to Claim 49, wherein the predetermined kinase activity is less than 10,000 Relative Light Units per mg of kinase when measured in the presence of luciferin/luciferase by a luminometer.

51. A method according to Claim 50, wherein the predetermined kinase activity is less than 1000 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

52. A method according to Claim 51, wherein the predetermined kinase activity is less than 100 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

53. A method according to Claim 52, wherein the predetermined kinase activity is less than 10 Relative Light Units per mg kinase when measured in the presence of luciferin/luciferase by a luminometer.

54. A method according to any of Claims 35-53, wherein the predetermined reduction in kinase activity is equal to or greater than a 6-log reduction.

55. A method according to Claim 54, wherein the predetermined reduction in kinase activity is equal to or greater than a 7-log reduction.

56. A method according to Claim 55, wherein the predetermined reduction in kinase activity is equal to or greater than an 8-log reduction.

57. A method according to Claim 56, wherein the predetermined reduction in kinase activity corresponds to at least a 6-log reduction in the amount or concentration of kinase.

58. A method according to Claim 57, wherein the predetermined reduction in kinase activity corresponds to at least a 7-log reduction in the amount or concentration of kinase.

59. A method according to Claim 58, wherein the predetermined reduction in kinase activity corresponds to at least an 8-log reduction in the amount or concentration of kinase.

60. A method according to any of Claims 35-59, wherein the predetermined reduction in kinase activity corresponds to a reduction in Relative Light Units of at least 900,000 RLU.

61. A method according to Claim 60, wherein the predetermined reduction in kinase activity corresponds to a reduction in Relative Light Units of at least 990,000 RLU.

62. A method according to Claim 61, wherein the predetermined reduction in kinase activity corresponds to a reduction in Relative Light Units of at least 999,000 RLU.

63. A method according to Claim 62, wherein the predetermined reduction in kinase activity corresponds to a reduction in Relative Light Units of at least 999,900 RLU.

64. A method according to Claim 63, wherein the predetermined reduction in kinase activity corresponds to a reduction in Relative Light Units of at least 999,990 RLU.

65. A method according to any of Claims 35-64, wherein the confirmed reduction in the amount or activity of the biological agent is at least a 6-log reduction.

66. A method according to Claim 65, wherein the confirmed reduction in the amount or activity of the biological agent is at least a 7-log reduction.

67. A method according to Claim 66, wherein the confirmed reduction in the amount or activity of the biological agent is at least an 8-log reduction.

68. A method according to any of Claims 35-67, comprising measuring kinase activity prior to treating the sample and after treating the sample.

69. A method according to any of Claims 35-68, comprising treating the sample at 80°C for at least 10 minutes prior to measuring the residual activity of the kinase.

70. A method according to any of Claims 35-69, wherein measuring the residual activity of the kinase comprises adding a substrate comprising ADP to the residual kinase and measuring formation of ATP.

71. A method according to any of Claims 35-70, comprising continuing the treatment until the residual kinase activity or the reduction in kinase activity corresponds to a confirmed reduction in the amount or activity of the biological agent of at least 6 logs.

72. A method according to Claim 71, comprising continuing the treatment until the residual kinase activity or the reduction in kinase activity corresponds to a confirmed reduction in the amount or activity of the biological agent of at least 7 logs.

73. A method according to Claim 72, comprising continuing the treatment until the residual kinase activity or the reduction in kinase activity corresponds

to a confirmed reduction in the amount or activity of the biological agent of at least 8 logs.

74. A method of correlating the reduction in the amount or activity of a contaminating biological agent in a sample, wherein the biological agent is selected from the group consisting of bacteria, viruses, spores, proteins, peptides and prions, with the thermostable kinase activity of an indicator according to any of Claims 17 or 19-27, comprising:

- (i) preparing a sample containing a defined amount of the biological agent and a sample containing a defined amount of the kinase;
- (ii) subjecting the sample(s) to a treatment comprising exposure to one or more of a selected pH, pressure, enzyme, detergent, chemical sterilant or gas-phase sterilant;
- (iii) measuring the residual activity of the kinase and optionally calculating the reduction in kinase activity;
- (iv) measuring residual amount or activity of the biological agent and optionally calculating the reduction in the amount or activity of the biological agent;
- (v) repeating steps (i) to (iv), wherein at least one of the treatment parameters is changed.

75. A method according to Claim 74, wherein the biological agent is an infectious biological agent and the treatment reduces the infectious activity of the agent.

76. A method according to Claim 74 or 75, wherein the biological agent is a transmissible spongiform encephalopathy.

77. A method according to any of Claims 74-76, wherein the treatment parameter comprises one or more of time, temperature, pH, pressure, protease concentration, and concentration of sterilant or detergent.

78. A method according to any of Claims 74-77, wherein
the treatment comprises heating the sample(s) at a temperature of between
50-140°C, preferably 134-138°C;
the treatment parameter is time;
and wherein steps (i) to (iv) are repeated by subjecting the sample(s) to said
treatment for periods of 1, 5, 10, 20, 40 and 60 minutes.

79. A method according to any of Claims 74-78, wherein
the treatment comprises exposing the sample(s) to a pH of 9-14, preferably
about pH 12;
the treatment parameter is time;
and wherein steps (i) to (iv) are repeated by subjecting the sample(s) to said
treatment for periods of 1, 5, 10, 20, 40 and 60 minutes.

80. A method according to any of Claims 74-79, wherein
the treatment comprises exposing the sample(s) to a protease at a
concentration of 0.5-2 mg/ml, preferably 1mg/ml;
the treatment parameter is time;
and wherein steps (i) to (iv) are repeated by subjecting the sample(s) to said
treatment for periods of 1, 5, 10, 20, 40 and 60 minutes.

type of format therefore offers remarkable sensitivity for the detection of molecules using binding species linked to adenylate kinase (AK), ~~as described in WO 02/053723.~~

5 Use of a kinase, e.g. AK, coupled to bioluminescent detection has a number of other significant advantages. The assay gives a direct relationship between enzymatic activity and light production over a much larger range than other comparable assay formats. Thus whilst an assay using a traditional reporter enzyme such as horseradish peroxidase or alkaline phosphatase will give a
10 proportional response over 5-6 log dilutions, the AK-luciferase assay can provide a dynamic range of at least 8 logs. As direct indicators this makes them especially useful for processes which require a level of inactivation greater than the standard 6-log range as the signal can be made to be meaningful across the whole range of the assay, something that would not be
15 possible using other assay formats. This is particularly relevant for TSE inactivation where, in a worse case scenario, as many as 8-logs of infectivity may be present on the surface of a surgical instrument, assuming the presence of 1mg brain tissue at a level of up to 10^8 TSE infectious units per mg. Under these circumstances an indicator of the invention, providing an 8-
20 log range of signal is particularly valuable.

Given the type of processes for which a TSE indicator is required a high level of both thermal and physical stability is preferred. In an example below, the properties of a range of AK enzymes from thermophilic organisms were
25 compared. Even AKs from thermophilic organisms such as the indicator strain *B.stearothermophilus* lose the majority of their activity at relatively low temperatures. For a kinase-based indicator to be included in e.g. an autoclaving cycle, a significantly greater degree of thermostability, such as that demonstrated by the enzymes from the *Sulfolobus* species or *Pyrococcus*
30 *furiosus*, is used.

A number of additives and changes to formulation that increase the stability of an enzyme, e.g. a kinase, to heat inactivation will be known to those familiar with the art. The thermostable kinases used in embodiments of this invention